[Proc. Roy. Soc. Victoria, **48** (N.S.), Pt. II., 1935.]

Art. X.—*Notes on the organisms causing Brown Rot of Citrus Fruit in Victoria, Australia (Phytophthora citrophthora* (Sm. & Sm.) Leon & *P. hibernalis* Carne).

By E. I. McLENNAN.

[Read 10th October, 1935; issued separately, 1st June, 1936.]

Brown rot affecting Citrus fruits (oranges, grape-fruit, cumquots and mandarins) was first recorded for Victoria, Australia, by Brittlebank(1), the fungus concerned was identified by him as similar to *Pythiacystis citrophthora* Smith and Smith. This record was not accompanied by any description or figures of the pathogen. Leonian (2) has since transferred this fungus to the genus *Phytophthora,* and it is now generally known as *Phythophthora citrophthora* (Smith and Smith) Leonian. Cole (3) stated that lemons appeared to be immune from attack although growing in close proximity to diseased orange fruit. In 1925 Carne (4, 5, 6) described the organism which he believed to be responsible for Citrus brown rot throughout Australia. He considered it to be a new species of *Phytophthora,* named by him *P. hibernalis.** He states (5) "the disease in Victoria agrees in field symptoms more closely with the Western and South Australian disease than it does with the Californian. In the absence of any detailed mycological evidence to the contrary the writer considers that he is justified in regarding all Citrus brown rot in Australia as being due to *P. hibernalis.*" In 1928 (6), however, he recorded *P. citrophthora* as undoubtedly present in Western Australia although he suggested that its occurrence is apparently rare.

A laboratory examination of Victorian lemon and orange fruits attacked by brown rot has been made during 1931, 1932, and 1933. Specimens were obtained from various sources in the State and the associated pathogen isolated on numerous occasions. The results show that both *P. citrophthora* and *P. hibernalis* are present in Victoria, that both cause practically identical symptoms on the decaying fruit, and that the particular fungus causing the rot can only be identified after isolation, and detailed examination. *P. citrophthora* was obtained constantly in the autumn isolations (March, April, May, June), while *P. hibernalis* also appeared in the spring months (September, October), particularly from the

* Tucker (9) considers that Carne's organism is identical with *P. syringae* Klebs. Professor Fawcett, however, in correspondence suggests that there is some doubt about the culture obtained by Tucker as *P. syringae* Klebs. Before receiving this statement from Professor Fawcett an attempt was made to procure standard cultures of *P. syringae* from Baarn for comparison with *P. hibernalis;* however, although in all ten cultures of *P. syringae* were sent to me at different periods of the year not one culture was viable on arrival in Melbourne. *P. syringae* has a low temperature maximum for growth and evidently passage through the tropics killed this fungus.

orange. As brown rot of the fruit is more prevalent in Victoria in the autumn, *P. citrophthora* may be looked upon as the more important pathogen in this State.† (Text-fig. 1.)

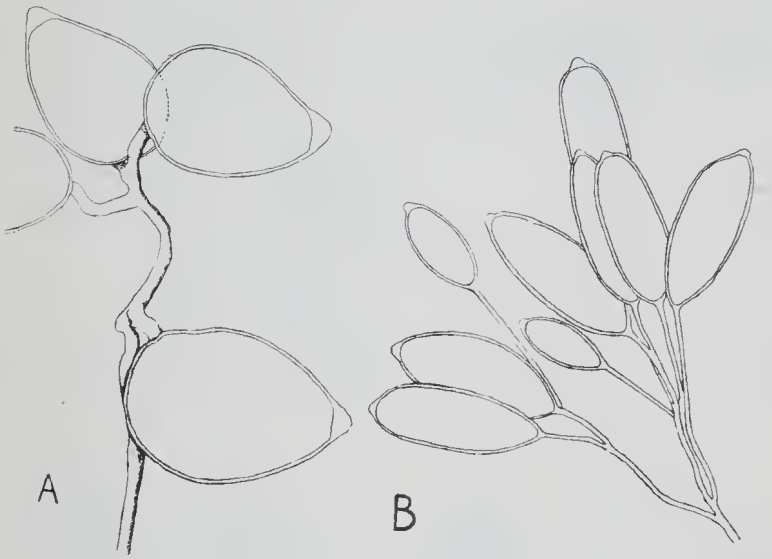


Fig. 1.—A. *Phytophthora citrophthora*—sporangiophore and sporangia. × 200.
B. *P. hibernalis*—sporangiophore and sporangia. × 200.

Comparison of the Cultural Characters of Australian Isolations with a Standard Culture of *P. citrophthora* (Sm. & Sm.) Leonian.

A culture of *P. citrophthora* (Sm. and Sm.) Leon. was obtained through the kindness of Dr. Waterhouse, N.S.W. It came originally from the University of Minnesota. At the same time he sent me a form which he had in culture, and which had been isolated by him from orange fruits from Queensland. These, as well as the Victorian isolations from lemon, were grown in culture, and the type of growth, and morphological characters of each were compared. On solid culture media, the Queensland and Victorian fungi appeared to be very similar, but they differed in some respects from *P. citrophthora* (American isolation) as is shown in the following table. Descriptions taken from cultures six days old.

† Brown rot of *Citrus* in N.S.W. is considered to be due to *P. hibernalis* Carne, as the following data supplied to me through the courtesy of Dr. Noble, May, 1932, show:—"The N.S.W. organism was first isolated in May, 1928, from orange fruits affected with brown rot. No detailed work has been attempted with it, but it corresponds very closely with Carne's description of *P. hibernalis* and with a subculture of his which we had through Dr. Waterhouse. The organism has since then been isolated readily and repeatedly in the autumn of each year, but there are no records of attempted isolations in the spring, the symptoms on the fruits usually being considered sufficient for diagnosis."

Medium.	*P. citrophthora.*	Victorian Isolation.	Queensland Isolation.
Potato — dextrose agar (as used by Carne (5))	Diameter of colony 6 cm. Aerial mycelium conspicuous. Culture silky in appearance progressing outwards by fan-like sectors. Edge fibrillose	Diameter of colony 4·75 cm. Aerial growth poor. By transmitted light the culture appeared granulated, the more opaque patches correspond with small areas of aerial mycelium. Two distinct concentric zones present, edge fibrillose	Diameter of colony 4·5 cm. Aerial growth poor, and culture similar to the Victorian isolation
Malt agar (Plate X, fig. 3)	Diameter of colony 4·5 cm. Margin of culture somewhat uneven as advancing over agar in fan-like manner. Silky-smooth growth, on a white background the culture appeared white, not sodden	Diameter of colony 4·5 cm. Culture expanding evenly over agar. Growth flat, appressed granular appearance extending from centre through a diameter of approximately 1·5 cm. on a white background, colony appeared sodden, almost buff-coloured	Identical with Victorian form
Oat agar ..	Diameter of colony 6 cm. Cobwebby aerial growth extending over colony except 0·5 cm. around the margin	Diameter of colony 6 cm. Very similar to *P. citrophthora*, but margin devoid of aerial mycelium not so distinct	Identical with Victorian form

When inoculated into sterile prune juice, a further difference between the standard cultures of *P. citrophthora* and the Australian isolations may be noted. Two to three weeks after an inoculum has been placed in a tube containing prune juice to a depth of 5 cm., the former grows through the medium and reaches the surface of the liquid, while the latter remains restricted in its growth, and only extends through approximately 2.5 cm., the upper portion of the liquid being free from fungal growth. (Plate IV., Fig. 1). This result has been noted repeatedly, and, together with the differences shown in their growth on solid media, suggests that the Australian form is not identical with the Californian organism, although morphologically they are indistinguishable, and agree in the type and manner of sporangial formation and lack of sexual organs.

Some Morphological and Physiological Features of *P. citrophthora* and Allied Forms Isolated from Citrus in Australia.

The general structural features of *P. citrophthora* have been described in detail by many workers, and my observations are in accord with the published accounts of this organism, with perhaps a few exceptions. Smith (7) illustrates the formation and escape of the zoospores from the sporangia of *P. citrophthora*. They are shown escaping directly from the sporangium. However, on numerous occasions in the present investigation, the formation of a vesicle by the sudden extrusion of the papilla,

and the passage of some of the zoospores into this structure has been observed. The vesicle remains intact, and is visible with careful focussing for an appreciable time; then, when distension has apparently reached its limit, the vesicle bursts and the zoospores actively swim away, continuing in motion under the coverslip for ten minutes or so, but they soon attach themselves to the glass, round off, and germinate immediately, by putting out one or more germ tubes. The outline of the zoospores in the sporangium is discernible before vesicle formation occurs. A similar type of germination of the sporangium occurred in the Australian isolations (Fig. 2). Although Smith and Smith (8) reported that no chlamydospores ever developed in cultures of *P. citrophthora*, these were reported by Tucker (9), and have been found by the writer in abundance, particularly in old liquid cultures. The majority were produced terminally, they were spherical, and measured on an average 28μ (Fig. 2H).

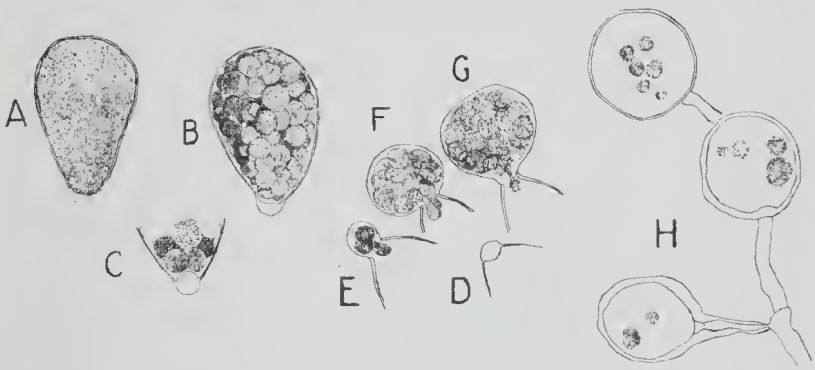


FIG. 2.—*P. citrophthora* (American strain) A-G sporangium at various stages of germination

A, at 12.25 p.m., B, 12.45 p.m., C, 1.5 p.m., D, E, F, G, at rapidly succeeding intervals, H, Chlamydospores from a prune juice culture set up April, 1932, examined September, 1932. × 200.

Tucker (9) discusses *P. citrophthora* and makes the following statement:—"The data show that the three isolations (*P. citrophthora*, Nos. 154, 221, and 222) are very similar in respect to the morphology of sporangia and chlamydospores, and in pathogenicity, but they do not reveal any character by which *P. citrophthora* may be separated from *P. palmivora*, some strains of which produce sporangia sparingly, and both sporangia and chlamydospores which do not differ much in size or form from those of *P. citrophthora*. The presence of a pedicel on fallen sporangia is another common character of both species. Examination of the temperature relations shows that the three isolations of *P. citrophthora* grew less rapidly at 30°C. than at

27.5° and 25°C. and no growth occurred at 32.5°C.; all three made some growth at 5°C. *P. palmivora* characteristically developed at 32.5°C. but not at 5°C., and grew most rapidly at 27.5° and 30°C. The differences in response to temperature are not large, but the constant behaviour of the isolations of *P. citrophthora* indicate that the species cannot be considered identical with *P. palmivora* . . . . . The differences in the abilities of the groups to grow at 32.5°C. and 5°C., the optimum growth of *P. citrophthora* at 25–27.5°C. and of *P. palmivora* at 27.5–30°C., indicate essential differences in the protoplasm of sufficient importance to justify the maintenance of *P. citrophthora* as a species."

Tucker goes on to suggest that *Phytophthora* spp. isolated from *Citrus* in various geographical regions may have been referred to *P. citrophthora* when a more careful investigation might reveal them to be *P. palmivora,* and in this connexion states that he has examined isolations from Porto Rico, Australia, Formosa, and the Philippines without finding one which agreed with the Californian fungus. The origin of the Australian form handled by Tucker is not given.

Since the isolations from *Citrus* made by the writer, although similar to one another, differed from the Californian isolation in some cultural characters they were next compared with isolations of *P. palmivora* obtained from the Centraalbureau voor Schimmelcultures, Baarn. One of these (No. 99) was isolated from cocoanut, another (No. 100) from *Cacao*, and both belong to the "rubber" group of *P. palmivora.* Malt agar and cornmeal agar plates were inoculated from young actively growing colonies of the Victorian fungus, the Queensland fungus, *P. citrophthora* (Californian isolation), and Nos. 99 and 100 (*P. palmivora*). The plates were prepared in triplicate for each form and were incubated at 5°C. and at 32°C. for five days.

Inoculum.	5° C. Diameter in mm.		32° C. Diameter in mm.	
	Oat.	Malt.	Oat.	Malt.
P. citrophthora	1·0	0·5	..	..
Victorian isolation	1·0	0·5	..	..
Queensland isolation	1·0	0·5	..	..
P. palmivora (No. 99) ..	..	..	25.0	18·0
P. palmivora (No. 100) ..	..	..	17.5	15·0

This experiment was repeated several times and in no case was any growth obtained with the Australian fungi on the plates incubated at 32°C. Since both Nos. 99 and 100 represented the

"rubber" group of *P. palmivora* in a later experiment, a culture of *P. palmivora* ("cacao" group) was substituted for 99, but exactly comparable results followed.

Those plates which showed no growth at either 5° or 32°C., when removed from the incubators, were left at room temperature (approximately 16°C.) for some days. The inocula were still viable, for growth commenced, and vigorous mycelial development occurred. For instance, on 31.10.34 several dishes inoculated with *P. citrophthora*, and the Victorian isolation, were placed in an incubator at 32°C. On 7.11.34 no growth had taken place. They were then removed, and placed at room temperature and, by 19.11.34, the mycelial mat measured 8.4 cm. in diameter.

As Tucker considers that the temperature relations of *P. citrophthora* and *P. palmivora* establish a constant difference between these two species, the Australian isolations should be regarded as a strain of *P. citrophthora*.

Adam (10) included *P. citrophthora* in a list of fungi isolated from oranges in cool store, and stated "the presence of the diseases, brown rot due to the fungus *P. citrophthora*, and the cottony mould *Sclerotina Libertiana*, should be noted. The possible serious consequences of these diseases in storage problems, as shown by experience elsewhere, demand that they should receive attention in the grove, &c." The fruit had been stored at temperature equivalent to 0°, 1.1°, 2.8°, 3.9°, and 4.4°C. In correspondence with Mr. Adam he informed me that the identification of the fungus he referred to as *P. citrophthora* was based on grounds which would not exclude the possibility of the fungus being *P. hibernalis* or indeed any other *Phytophthora* sp.

Further evidence that the Australian fungi should not be regarded as strains of *P. palmivora* was obtained when these isolations were opposed to strains from the "rubber" and "cacao" groups of *P. palmivora*. In the dishes where the "rubber" and "cacao" strains were mated, sexual organs typical of *P. palmivora* could always be obtained, but in no case did sex organs occur when the various Australian isolations were tried against both strains of *P. palmivora*.

Summary.

1. Isolations of a *Phytophthora* made from Citrus fruit affected with brown rot have been compared with a standard culture of *P. citrophthora* (Sm. & Sm.) Leon., and although certain cultural differences were noted, they are considered to be strains of this Californian fungus, and not *P. palmivora* as suggested by Tucker.

2. Both *P. citrophthora* and *P. hibernalis* were isolated in Victoria. The former pathogen, however, appears to be much commoner, and was the only species isolated from *Citrus* during the autumn months.

Bibliography.

1. Brittlebank, C. C.—The Leader, Melbourne, Victoria, 4th June, 1921.
2. Leonian, L. H.—"Physiological studies on the genus *Phytophthora*." *Amer. Journ. Bot.* xii, pp. 444-498, 1925.
3. Cole, C. F.—"A new *Citrus* disease—*Pythiacystis citrophthora*." *Jour. Agric. Vic.* xix., pp. 363-366, 1921.
4. Carne, W. M.—"*Citrus* disease," Brown Rot and Leaf Blight. *Journ Agric. W.A.*, I., pp. 519-522, 1924.
5. ————. "A brown rot of *Citrus* in Australia." *Journ. Roy. Soc. W.A.*, xii., pp. 1341, 1925.
6. ————. Additions to the plant diseases of S.-W. Australia. *Journ. Roy. Soc. W.A.*, xi., pp. 43-68, 1924-25, and xiv., pp. 23-28, 1928.
7. Smith, R. E.—"The brown rot of the lemon." *Ag. Expt. Sta., Univ. of Calif.* Publ., Bull. No. 190, 1907.
8. Smith, R. E., and E. H.—"Further studies on Pythiaceous infection of deciduous fruit trees in California." *Phytopathology* xv., pp. 389-404, 1925.
9. Tucker, C. M.—"Taxonomy of the genus *Phytophthora* de Bary." *Ag. Expt. Sta., Univ. of Miss.*, Res. Bull. 153, 1931.
10. Adam, D. B.—"Experiments in citrus fruit storage." *Journ. Dept. Ag. Vic.* xxi., pp. 307-317, 1923.

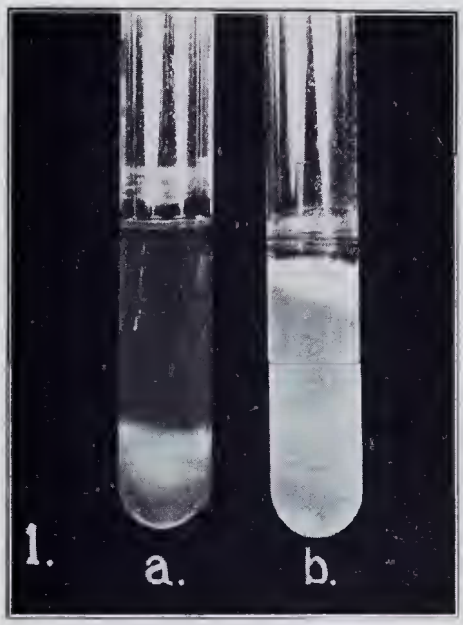
Explanation of Plate.

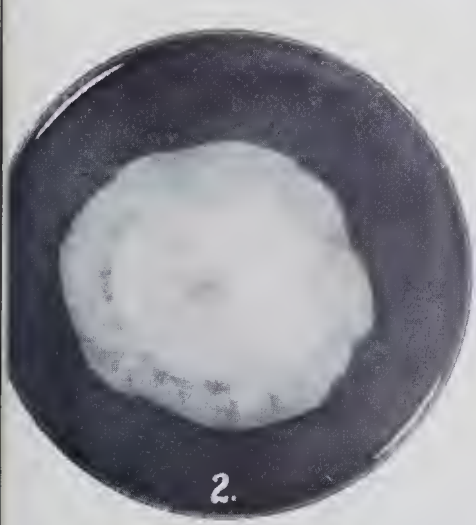
Plate X.

Fig. 1.—(*a*) Australian strain of *Phytophthora citrophthora* in prune juice, culture 3 weeks old.
(*b*) American strain of the same species, culture same age as in *a*.

Fig. 2.—*P. citrophthora*, American strain on malt agar.

Fig. 3.—*P. citrophthora*, Australian strain on malt agar.





Cultural Characters of Phytophthora citrophthora.